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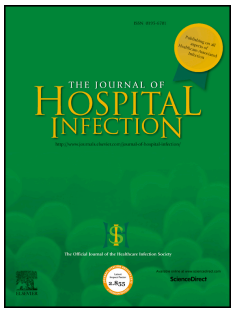

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Multidrug-resistant Gram-negative bacteria: a product of globalization

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SUMMARY

Global trade and mobility of people has increased rapidly over the last 20 years. This has had profound consequences for the evolution and the movement of antibiotic resistance genes. There is increasing exposure of populations all around the world to resistant bacteria arising in the emerging economies. Arguably the most important development of the last two decades in the field of antibiotic resistance is the emergence and spread of extended-spectrum β-lactamases (ESBLs) of the CTX-M group. A consequence of the very high rates of ESBL production among Enterobacteriaceae in Asian countries is that there is a substantial use of carbapenem antibiotics, resulting in the emergence of plasmid-mediated resistance to carbapenems. This article reviews the emergence and spread of multidrug-resistant Gram-negative bacteria, focuses on three particular carbapenemases – imipenem carbapenemases, *Klebsiella pneumoniae* carbapenemase, and New Delhi metallo-β-lactamase – and highlights the importance of control of antibiotic use.

Keywords:

Antibiotic resistance

Carbapenemases

CTX-M ESBLs

Extended-spectrum β-lactamases

Global trade

Multidrug-resistant Gram-negative bacteria

Introduction

Global background

There has been a massive increase in global trade over the last 20 years, especially with the rapidly emerging nations of China and India. In 2008 more than US$800 billion of

trade flowed between Asia and Europe and almost half the global trade in goods involved Europe.[1] This has had profound consequences for the evolution and the movement of antibiotic resistance genes. Of the top ten megacities (cities with ≥10 million population) eight are in Asia, the other two being Mexico City and New York. These megacities place huge demands on public health infrastructure, particularly in relation to sewage, drinking water, and overcrowding. In addition, the emerging economies are often heavy users of antimicrobials in both medicine and agriculture, which, combined with the deficits in public health infrastructure, has resulted in very high rates of resistance to antibiotics, especially among Gram-negative bacteria.

Global mobility has changed dramatically in the last 15 years. Travel by air has increased to the extent that, in 2012, >5000 billion revenue passenger-kilometres were recorded by the International Civil Aviation Organization.[2] Passengers carry Gram-negative bacteria in their bowel flora, particularly *Escherichia coli* and *Klebsiella* spp. Consequently there is increasing exposure of populations all around the world to resistant bacteria arising in the emerging economies. The role of the environment in the transmission of Gram-negative bacteria has been reviewed in detail elsewhere.[3] Unlike MRSA and enterococci, Enterobacteriaceae have a more fluid genome, mediated chiefly by the extensive carriage of conjugative plasmids that frequently carry antibiotic resistance genes as well as pathogenicity genes. The ability of antibiotic resistance genes to be transferred from environmental bacteria to medically relevant species of bacteria is well recognized.

Emergence of extended-spectrum β-lactamases (ESBLs)

Arguably the most important development of the last two decades in the field of antibiotic resistance is the emergence and spread of extended-spectrum β-lactamases (ESBLs) of the CTX-M group.[4] Careful work by a group in Paris has shown that CTX-M resistance genes are present on the chromosomes of at least three species of *Kluyvera*, environmental bacteria closely associated with the rhizosphere (the complex microbial community surrounding plant rootlets). The resistance gene is inducible when on the chromosome but, once mobilized on to a plasmid with insertion sequences ISEcp1 and IS903, becomes highly mobile among the Enterobacteriaceae.[5] CTX-M was first recognized in 1989 in an isolate from a cancer patient of *E. coli* which was resistant to third generation cephalosporins. It was located on an 85 kilobase conjugative plasmid and the enzyme was originally designated MEN-1.[6] Four major groups of genetically distinct but related genotypes of CTX-M have emerged: 1, 2, 25, and 9.[7] Of the genotypes within these groups, two have been immensely successful, i.e. CTX-M 15 and to a lesser extent CTX-M 14.[4] CTX-M 15 is the only genotype present throughout India. Bearing in mind the early recognition there of the ESBL phenotype

and the genotyping of strains from the early 2000s, it seems most plausible that this gene emerged in the Indian subcontinent and has then spread throughout the world.[8] CTX-M 14 was first described in Enterobacteriaceae from Guangzhou in Southern China in 1998.[9] The study indentifying CTX-M 14 observed a very high rate (≥35%) of ESBL production among *E. coli* in Guangzhou and, by reasoning similar to that applied to CTX-M 15 in India, it is most likely that CTX-M 14 emerged in China or an adjacent country during the mid-1980s, when locally produced cefotaxime was very widely used in hospital practice.

Spread of ESBLs

The movement of people around the world is a major consideration in the spread of multidrug-resistant (MDR) Gram-negative bacteria. Recent statistics for passenger movements at UK airports reveal that while the number of movements to or from the USA and Canada grew from 19.8 million in 2002 to 20.4 million in 2012, movements to or from India and Pakistan grew from 1.3 to 2.7 million over the same period, and to or from China and Hong Kong from 1.3 to 2.1 million. Total movements between the UK and the rest of the world grew from 39.5 to 50 million. Several studies have demonstrated the significance of this movement for antibiotic resistance. For example, Tham *et al.* found ESBL-producing bacteria in only two of 63 (3%) travellers returning to Sweden from European destinations, whereas they found ESBL producers in 50 of 138 (36%) of those who had travelled outside Europe.[10] The highest colonization rate was in those returning from India (11/14, 79%) closely followed by Egypt (19/38, 50%) and the Middle East (4/10, 40%). The genotypes of CTX-M identified in the travellers matched the regional distribution described by Hawkey and Jones.[4] All the ESBLs from travellers returning from India were CTX-M group 1 (most likely CTX-M 15), whereas all those returning from China were group 9 (most likely CTX-M 14). Egypt and Thailand had a mixture of groups 1 and 9, which is consistent with studies from those countries. Acquisition in these countries is at least partly attributable to the variable quality of sewage disposal and water treatment. In India, only 47% of households have a latrine.[11] Defecation in public places is a frequent occurrence, thus facilitating the spread of colonization among both local people and visitors. The situation in China is better, as 65% of the population have access to improved sanitation facilities (36% in India), but disposal of faeces can be of a variable standard especially in rural areas.[12] Even in those countries with level 3 treatment of sewage such as the UK, sewage treatment does not fully remove CTX-M-producing *E. coli*. A recent study from the English Midlands has shown that significant numbers of CTX-M-producing *E. coli* in treated effluent are discharged into water courses, where they may then be acquired by people during recreational activity in what appears to be a perfectly clean river, and also by livestock.[13] There is a paucity of data from

many countries in Asia on resistance rates, but the SMART study (which samples *E. coli* and *Klebsiella* spp. causing significant intra-abdominal infections across a range of countries throughout the world and subjects them to standardized antimicrobial susceptibility testing and characterization) shows some interesting contrasts in different Asian countries. The data from 2008 showed an ESBL-producing *E. coli* rate for China of 59.1% and for India of 61.2%.[14] This contrasts with a rate of 22% in Hong Kong and 2.9% in Malaysia, which have better public health infrastructures as well as reasonably controlled antimicrobial prescribing in both human and animal medicine. Data on ESBL carriage rates in the community are even scarcer. Recently all of the available studies were summarized by Woerther *et al.*[15] Data on individuals with or without healthcare contact in the community are completely lacking from India. However, there are three studies of ESBL producers in healthy individuals in the community in China: Tien *et al.* reported a prevalence of ESBL-producing *E. coli* of 7% among 170 elderly people in Shenyang; by contrast, Li *et al.* reported a prevalence of 50% among 109 individuals in Fuzhou; and Zhong *et al.* found a prevalence of 51% in 567 healthy individuals with no healthcare contact in Hunan Province.[16–18] The high rate of carriage of CTX-M ESBLs in some countries is reflected in the carriage rate in those individuals in European countries with connections to those countries. Wickramasinghe *et al.* demonstrated this effect by showing that residents of Birmingham, UK, whose names indicated a global origin in either the Middle East or South Asia, had a CTX-M ESBL faecal carriage rate of 23%, whereas those whose names indicated European origin had a carriage rate of 8%.[19] There was also a statistically significant association of carriage of CTX-M 15 among the Middle Eastern/South Asian individuals. A similar effect has been noted recently in Paris, where CTX-M-positive clinical isolates were much more prevalent in those whose birthplace was outside of mainland France.[20]

A consequence of the very high rates of ESBL production among Enterobacteriaceae in Asian countries is that there is a substantial use of carbapenem antibiotics, which has resulted with time in the emergence of plasmid-mediated resistance to that family of drugs. Of the five widely encountered carbapenemase genes – IMP, KPC, NDM, VIM, and OXA-48 – this review concentrates on IMP, KPC, and NDM.

IMP carbapenemases

IMP carbapenemases, the first plasmid-mediated transferable carbapenemases to be recognized, emerged in Japan in the 1990s.[21] The carbapenemases, like the CTX-M ESBL enzymes, have a range of genotypes that are given sequential numbers (e.g. IMP-1, IMP-2, etc.), with each new carbapenemase differing from previously described genotypes by at least one amino acid. The first genotype described, IMP-1, is the most widely encountered

carbapenemase in Japan. This emerged and spread rapidly among very different species of Enterobacteriaceae – *Acinetobacter* and *Pseudomonas* spp. Nine other genotypes were recognized between 1995 and 2001. One of those was IMP-4, first described from a single isolate of *Citrobacter youngae* from Guangzhou in Guangdong Province of Southern China.[22] IMP-4 was encoded on a cassette in a class 1 integron on a large plasmid and was transferrable to *E. coli*. An interesting feature of both IMP and VIM metallo-β-lactamases in class 1 integrons is their ability to be expressed at high level in different hosts, and to be mobilized from one strain to another and one species to another.[23] Fascinatingly, almost simultaneous with this first description of IMP-4 in *Citrobacter youngae*, there was an outbreak in Hong Kong of *Acinetobacter baumannii* carrying the IMP-4 carbapenamase.[24] There were no further reports of IMP-4 until 2005, when there was a large outbreak in Melbourne, Australia, predominantly in *Serratia marcescens* but also in *Pseudomonas aeruginosa*, which subsequently spread to seven different species of Enterobacteriaceae and became endemic in eastern Australia.[25] Almost simultaneously Sydney experienced an outbreak of hospital cross-infection with Enterobacteriaceae producing IMP-4 in the same integron cassette array in which three other genes, *qacG2*, *aacA4* and *catB3*, were also present within the class 1 integron.[26] Interestingly the integron was found in a different genetic context and on a different plasmid from the original isolate from Guangzhou, suggesting movement either by homologous recombination or a CR1-associated mobilization. IMP-4 continues to be a problem within Eastern Australia. Following the initial reports in 2000, it was not until 2009 that IMP-4 was reported again (along with IMP-8 and KPC) in China, where it emerged as the most prevalent carbapenemase in a nationwide surveillance study involving 16 teaching hospitals.[27] More recent studies report the widespread occurrence of IMP-4 in southwest China, where a 2009–2010 study in a 3000-bed hospital in Chongquing revealed 26 isolates of carbapenem-resistant Enterobacteriaceae of which 18 produced IMP-4 and one produced KPC.[28] Interestingly, 16 of the carbapenemases were in *Klebsiella pneumoniae*. A study from Fujian Medical University Union Hospital of faecal carriage among 303 randomly selected patients between November 2011 and January 2012 revealed a carriage rate of carbapenemase-producing Enterobacteriaceae of 2.6%.[29] Four isolates produced IMP-4, four produced KPC and one of the KPC isolates also produced NDM-1. In Europe and North America, IMP carbapenemases are currently rare, but their widespread presence in both China and Australia suggests that this carbapenemase may prove to be a problem in the future.

KPC carbapenemase

Arguably one of the most widely distributed carbapenemases in the world is *K. pneumoniae* carbapenemase (KPC). It was first described in North Carolina, USA, in 1996, and re-emerged in New York City in the early 2000s, causing extensive outbreaks in intensive care units in the Brooklyn and Bronx districts.[30] KPC-1 and KPC-2 are identical: the initial difference was attributed to a single base pair error in sequencing and KPC-2 has been adopted as the correct DNA sequence. A number of other genotypes are recognized but none are as prevalent as KPC-2. KPC-3 has an enhanced activity against ceftazidime compared to cefotaxime. They are all serine-active β-lactamases which confer broad-spectrum resistance to all β-lactams except to high levels of temocillin. They are poorly inhibited by all the well-known β-lactamase inhibitors, but the new β-lactamase inhibitor, avibactam, when combined with ceftazidime shows excellent inhibitory activity against serine-active carbapenemases (KPC and OXA-48) as well as ESBLs. When KPC emerged, it was confined to ST-258 sequence type *K. pneumoniae* in the USA but emerged contemporaneously in *Pseudomonas aeruginosa* in Columbia and Puerto Rico. In the USA it was initially (in 2005) confined to the eastern seaboard with one or two states in the mid-west being affected, but the spread across the USA since then has been rapid. Data from the Centers for Disease Control and Prevention, Atlanta, on the situation in February 2014 showed that every state except Alaska and Idaho was affected. In many long-term care facilities, KPC is a considerable problem and is found in other Enterobacteriaceae. The global threat of KPC is exemplified by the experience in Israel. Because of a substantial problem since the late 1990s with CTX-M 2 ESBLs thought to have been imported by travellers from South America, carbapenems were heavily used. This created the ideal environment for the spread of KPC-producing *K. pneumoniae* that had likely been introduced to Israel by visitors from New York. First observed in Tel Aviv in late 2005, within two years it had subsequently spread to healthcare facilities throughout Israel.[31]

Greece has also had a substantial problem with KPC for a number of years, and, very recently, Italy has moved from a situation where carbapenemase genes were carried by less than 5% of *K. pneumoniae* isolates to one where, in 2011, they were carried by 25–50% *K. pneumoniae* isolates.[32,33] This might be linked to Italy's position as one of Europe's heaviest prescribers of carbapenems and lowest users of alcohol hand-rub gel.[33] In China, KPC appears to be frequently co-carried with IMP-4 but it is not as prevalent, and isolates solely producing KPC are recognized. Qi *et al.* at Zhejiang University first drew attention to the KPC problem in a study of carbapenemase genes in 95 isolates of *K. pneumoniae* from 13 different hospitals across five provinces in Eastern China.[34] All isolates are found to carry KPC-2 and many had the ST-11 sequence type (a single locus variant of ST-258), but other sequence types were identified and pulsed-field gel electrophoresis revealed considerable

diversity of the host bacteria, suggesting extensive transmission via plasmids into different backgrounds. More recently, KPC has also been recognized in *E. coli* and in other locations within China.[35]

NDM carbapenemase

In 2008, a new metallo-carbapenamase only distantly related to the IMP and VIM genes was identified in a Swedish patient of Indian origin who had returned to India for medical treatment and then been admitted to hospital in Sweden.[36] Metallo-carbapenemase-producing *K. pneumoniae* was isolated from his urine, and *E. coli* carrying the same metallo-β-lactamase gene was isolated from his faeces. The carbapenemase, New Delhi metallo-β-lactamase (NDM), was named after the location of his recent hospital care in India. Later sporadic findings of this new NDM gene in the UK could in at least 50% of cases be linked to receiving hospital care in India, Bangladesh, or Pakistan.[37] India is a frequent destination for medical tourism from the UK.[38] Data on the distribution of NDM in South Asia are scarce, but NDM is probably one of the most widely distributed carbapenemases in South Asia, along with OXA-48, which can be difficult to detect phenotypically. NDM has also spread to many other parts of the world, generally, as in the UK, as sporadic importations. Carbapenem-resistant *Klebsiella* spp. and to a lesser extent *E. coli* are increasingly documented in hospital facilities in India: for example, Saleem *et al.* have described increasing carbapenem resistance in *K. pneumoniae* causing late-onset sepsis in a neonatal intensive care unit in Karachi. Whereas, before 2010, isolates of *K. pneumoniae* from babies with late-onset sepsis were only sporadically resistant to imipenem, in 2010, 23% (6/26) of the isolates were imipenem resistant and, by 2011, 72% (13/18) were imipenem resistant.[39]

Carbapenemase-producing Enterobacteriaceae in the UK

In the UK, all five major groups of carbapenemases, as well as rarer enzymes, have been reported by the reference laboratory. The UK picture is dominated by KPC, but most KPC-producing isolates were either from screening samples or from clinical specimens in northwest England. The next most frequently seen carbapenemase is NDM, which is distributed across the UK but with higher numbers in the London region. OXA-48 is also frequently encountered and many of these are attributable to episodes of cross-infection, particularly in intensive care units. I believe that the UK will see an increasing pressure in the future from imported NDM, which may, if not dealt with vigorously, cause local problems. The currently endemic KPC in northwest England is being controlled and may yet be eradicated, but we will experience further pressure from imported cases. In my view, the UK should be especially alert to future importations of NDM-1 and IMP-4 from Asia. Moreover, OXA-48 may well become locally endemic, as its detection is extremely problematic.

The environment and Gram-negative resistance

Faecal carriage by humans is a major source of MDR Gram-negative bacilli within the hospital environment and can be selected by antimicrobial therapy. Once MDR Gram-negative resistance genes become widespread in the community, the opportunity exists for bacteria to be more widely disseminated through the environment and establish a cyclical pattern of distribution.[3] River sediment has recently been shown to be a substantial reservoir of antibiotic resistance genes.[40] Using a metagenomic approach, the authors demonstrated that these were present both in culturable and unculturable bacteria. Even the use of level 3 sewage treatment in the UK does not prevent significant numbers of CTX-M-producing Enterobacteriaceae being released into the aquatic environment.[13] They can then be acquired by people engaged in water sports, as well by farm animals, which may explain the acquisition by dairy herds of CTX-M 14 and 15, the two most prevalent types in humans.[41] Once in the human food chain, the cycle can be completed (Figure 1). World production of poultry and pig meat has been increasing rapidly. Statistics from the UN's Food and Agriculture Organization in 2011 show that China produced 17.6 million tons of poultry meat and Europe produced 16.4 million tons.[42] Production of pig meat was 62.0 million tons in China, 26.8 million tons in Europe and 12.1 million tons in North and South America. Chinese production of pig meat represents 5% of total world production. The use of antibiotics in animal husbandry in China and many other Asian countries is substantial, although detailed data are not generally available. Xinhua news agency has published a report from the Ministry of Health and National Antibacterial Resistance Investigation Net on the production of antibiotics in China in 2007.[43] In all, 210,000 tons of antibiotics were produced that year, of which 30,000 tons were exported and 46% was used in food animals. The total agricultural and medical consumption of antibiotics thus amount to 136 grams per person per year. By contrast, antibiotic consumption England in 2013 was 18 grams per person per year. Several factors driving antimicrobial use in medicine in both China and India need to be addressed urgently to reduce the overall selection pressure.

Aquaculture, including fish farming, is another aspect of environmental and agricultural usage of antibiotics that gives cause for concern. In Scandinavian countries, the use of antibiotics in aquaculture has been greatly reduced by the development of vaccines and alternative farming methods, but this is not true in warm water aquaculture where antimicrobials, especially quinolones, are extensively used. Chinese aquacultural production has risen from six million tons in 1990 to 32 million tons in 2008, when it represented 62% of world production.[42] The first study of antibiotic-resistant bacteria in farmed fish in China was published by Jaing *et al.* in 2012.[44] They studied farmed fish from 15 market outlets across

Guangdong province (the Chinese province with the greatest production of farmed fish) and looked for ESBLs and plasmid-mediated quinolone resistance genes. *Escherichia coli* colonies were taken from non-selective media (one colony per sample) from the gut contents of each of 20 fish from 15 different markets. Eighty were ciprofloxacin resistant, of which 30 out of 80 carried *qnrB* genes and 16 out of 30 isolates carried *qnrS*. Other significant quinolone resistance-determining genes [*qnrD and aac(6')-Ib-cr*] were also found in the isolates, as well as a small number of ESBL genes (^{bla}CTX-M-14 and ^{bla}CTX-M-79), the most frequently occurring genotypes in the Chinese population.[44]

Future control of MDR Gram-negative bacilli around the world

Effective control requires good surveillance, and it is somewhat disquieting that, in the 2014 antimicrobial resistance report on surveillance from the World Health Organization, only 50% of member states returned data sets on nine key 'drug–bug' combinations.[45] Returns were highest in European states (38/53, 72%), but low in the Southeast Asian states (6/11, 55%). No data were available for the 2013 data collection exercise from either India or Indonesia, countries of populations of 1.2 billion and 400 million, respectively. Data are also lacking from many African countries. If we are to combat the rise and global spread of MDR Gram-negative bacilli, all countries must contribute to accurate and timely surveillance. Surveillance is information for action: the only country yet to have successfully controlled carbapenemase-producing Enterobacteriaceae is Israel, which launched a concerted programme of screening and cohort isolation.[46] This level of intervention will be difficult to introduce in many parts of the world where there are weak laboratory facilities, poor infection control, and widespread prescribing of antibiotics. The threat for the future appears to come largely from the Asian region, where increasing wealth and intensification of both medical practice and farming results in increased usage of antibiotics. China's introduction of controls of antibiotic consumption is extremely welcome. Activity in India appears modest thus far. As African nations become wealthier and more agriculturally intensive, we may expect the same pattern to be repeated. If we are to avoid squandering the valuable resource of antimicrobials, we must act globally.

Conflict of interest statement

None declared.

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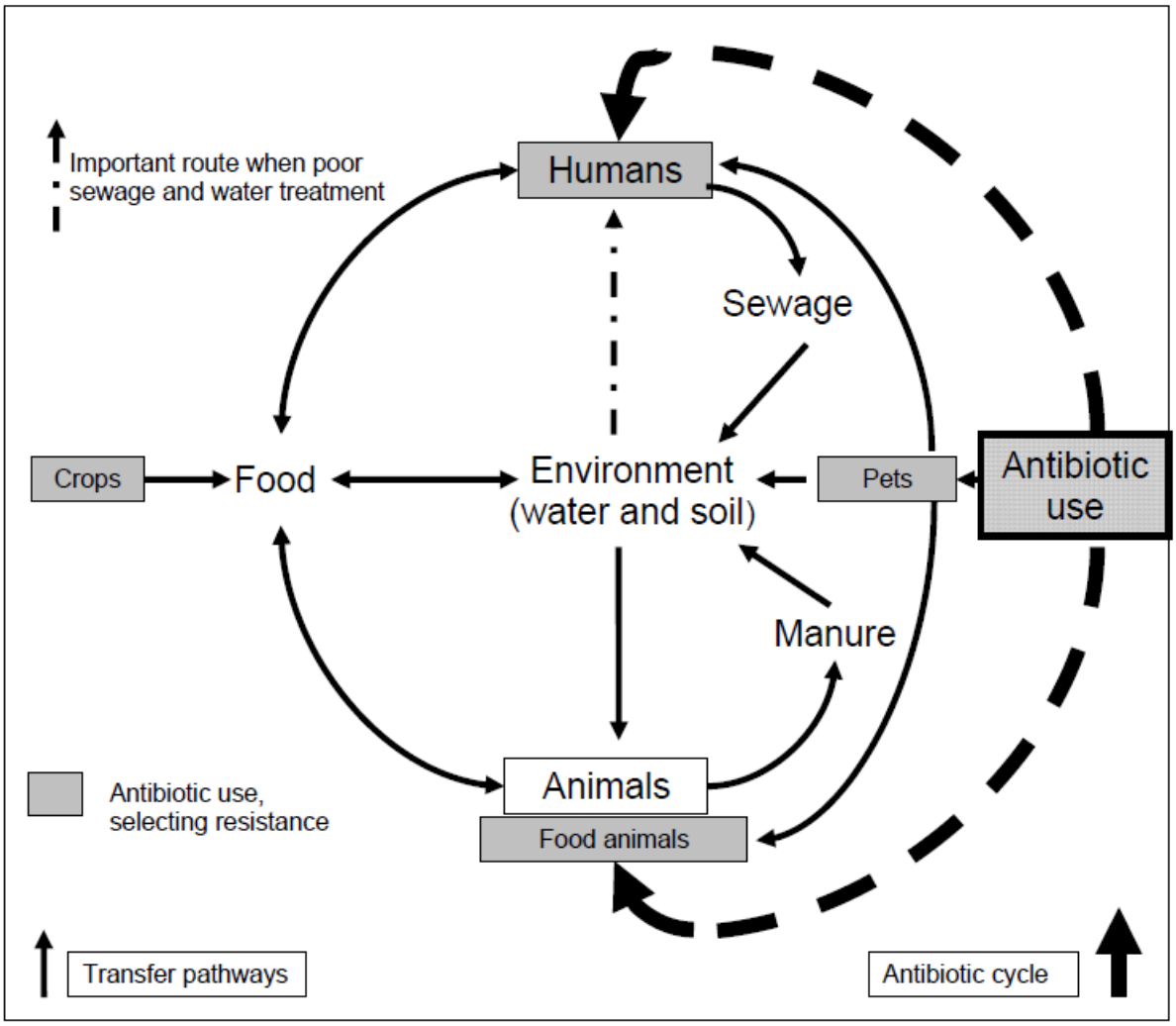

Figure 1. Principal transfer pathways for antibiotic resistance genes in humans, animals, food, and the environment. (Reproduced with permission from The Joint Working Group of Defra's Antimicrobial Resistance Co-ordination (DARC) and Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infections (ARHAI). ESBLs – a threat to human and animal health? https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/215180/dh_132534.pdf [accessed 16.01.15]).